**Special Issue:**

Emerging and Re-emerging Animal Health Challenges in Low and Middle-Income Countries

Anthelmintic Effect of Silver Nanoparticles of *Citrullus colocynthis* on *Leishmania tropica* Isolated from Pet Animals *In Vitro*

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Abstract | The current study aim to prepare several types of extracts (aqueous, hot, cold, alcoholic, and nano) from the colocynth plant to determine the inhibitory effect of these extracts on the number and vitality of the *Leishmania tropica* promastigotes in vitro, The results shown that even at low concentrations (0.5 mg), the colocynth plant's green-produced silver nanoparticles demonstrated strong antileishmanial activity against *Leishmania tropica* promastigotes during a 24-hour incubation period. The development of the parasites was significantly inhibited both in number and viability of promastigote that treated with green AgNPs of *Citrullus colocynthis* comparing with control. It also caused killing the promastigote on third day after treatment in all concentration of green nanoparticles of plant (0.5, 1, 1.5 and 2) grams and shown best results in lowering viability in two days after treatment, While the alcoholic extract had an greatest significant inhibitory effect at concentration of 2 grams on the eighth day of incubation, killing of the 100% of the live parasites was observed comparison with the control group No any significant effect of the hot and cold aquatic colocynth extracts was observed on the vitality of promastigotes. Only the tenth day of treatment at concentration of 2 grams had a slight significant decrease in the vitality of the promastigotes.

Keywords | Silver nanoparticles, *Citrullus colocynthis*, *Leshmina tropica*, Pet animals

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INTRODUCTION

Leishmaniasis is a significant protozoal disease affecting humans and animals, characterized by diverse clinical manifestations. It is considered one of the world's most neglected vector-borne diseases (Alvar *et al.*, 2012; Desjeux, 2004).

It can be caused by a number of *Leishmania* species. The bulk of these organisms are mostly found in animals; however some are mostly maintained in humans. The majority of the species in the latter group are zoonotic. Leishmaniasis is spread by sandflies and can be difficult

to avoid. Many of the drugs used to treat the illness are difficult to get outside of endemic areas or have severe adverse effects (Aoun and Bouratbine, 2014).

While several organisms have been identified, including *L. major*, *L. tropica*, *L. mexicana*, *L. colombiensis*, *L. amazonensis*, *L. braziliensis*, *L. panamensis*, *L. guyanensis*, *L. peruviana*, and *L. major*, it is most probable that *L. infantum* is the source of the majority of clinical cases. Some species are occasionally encountered, such as *L. major* and *L. tropica*. *L. infantum*, *L. mexicana*, *L. venezuelensis*, *L. braziliensis*, and *L. amazonensis* are among the species that are currently known. when felines have leishmaniasis. Leishmaniasis

cases are also sometimes observed in equids; in certain cases, *L. infantum*, *L. braziliensis*, and *L. martiniquensis* (formerly named *L. siamensis*) have been recorded as the causal organisms. Although the only known natural host of *L. enriettii* is the guinea pig, experimentally infected hamsters also get minor lesions (Dahroug *et al.*, 2010; Lemma *et al.*, 2009).

Iraq was home to an endemic case of cutaneous leishmaniasis (CL) (Pringle, 1957). Leishmaniasis was formerly thought to be the ninth parasite infection on Earth (WHO, 1990). In Iraq, the disease was thought to be becoming more prevalent in the focus district as well as in the rural and semi-desert regions overall (Sukkar, 1978).

Leishmaniasis is becoming more common; certain endemic zones have reported a 50% increase in recent years. Currently, 88 countries have an endemic case of leishmaniasis, putting 350 million people at risk (WHO, 2000). *Leishmania* important species were identified as the primary source of cutaneous leishmaniasis patients in Iraq, namely in the Basrah governorate (Jarallah, 2003).

Many different types of secondary metabolites, including flavonoids, alkaloids, terpenoids, and tannins, are abundant in plants and have been shown to have antibacterial effects *in vitro* (Cowan, 1999).

Numerous bioactive substances found in plants can quickly and effectively kill parasites via a variety of methods, hence lowering the likelihood that they will become resistant to anthelmintic drugs. In addition to being readily biodegradable and environmentally benign, plants bioactive chemicals also have a lower potential for bioaccumulating in animal tissues and the surrounding environment (Delfin *et al.*, 2017).

Traditional medicine exists in all civilizations in some capacity; other classifications, based on cultural differences, including Chinese, African, and Asian medicine (Al-Rahbi, 2000). Many alternative treatment strategies have been proposed; screening medicinal plants for antileishmanial characteristics is one especially helpful strategy for finding new active components (Iwu *et al.*, 1994). The objective of this study was synthesis of silver nanoparticles using aqueous *Citrullus colocynthis*, a natural component.

Additionally, the study aim to determine the anthelmintic activity of the synthesis nanoparticles on *Leishmania tropica* isolated from pet animals *in vitro*. Finally, we aimed to compare the results of the aqueous and alcoholic and nano extract of *Citrullus colocynthis*.

LEISHMANIA STRAIN

The Iraqi strain of *L. tropica* utilized in this investigation was identified from ulcerative lesions on the skin of dogs with cutaneous leishmaniasis. After clinically examination of suspected CL lesions from veterinary clinics in rural village to the strain was confirmed as reported earlier by Alusi and Sulman (1979). To create smears, aspirate material from the lesion's edge was spread out over a slide. Leishman's stain was applied to the produced smears in order to identify Leishman bodies and inspect them using a light microscope's oil immersion objective.

The total DNAs were extracted from the positive stained smears of seven CL lesions. After that, DNA samples were quantitatively measured using NanoDrop (Thermo Scientific) and purified using the Qiagen kit (QIAquick PCR Purification Kit, Germany) in accordance with the manufacturer's instructions before being diluted to a final concentration of 50 ng/μL. Before using for molecular detection, each sample of 100 microliters of DNA was rinsed and kept at -20°C.

MOLECULAR PROCEDURES

By analyzing the 18srRNA genes, the *Leishmania*-specific ribosomal was detected in the DNA samples using PCR amplification. The amplification process took place in a 25 μl master mix volume that contained 5 μl of Taq PCR Premix Bioneer, Korea kit, 10 picomols/μ(1μl) Forward primer, and 10 picomols/μ(1μl) 1.55 μl of reverse primer DNA, 16.50 μl of distill water, and 45 μl of final volume in a tube. As reported by to Schonian *et al.* (2001). Initial denaturation and annealing has been found to be the ideal condition; multiple experiments were conducted to determine this condition; gradient PCR was used to vary the temperature for each sample in order to select the ideal condition; the concentration of DNA template was also varied between 1.5-2μl. After the PCR tubes holding the amplification mixture were placed in the thermal cycler, the amplification procedure was initiated. The optimal conditions for 18srRNA gene amplification were found at 95°C for initial denaturation, 95°C for denaturation, 55°C for annealing, and 72°C for extension, respectively. Following red staining at 5 volts per cent for 1:30 hours in 1X TBE buffer, the expected fragment of the 18 srRNA gene, approximately 500 bp, was successfully amplified for *Leishmania tropica*. This was verified by the electrophoresis analysis. The PCR products were separated by 1.5% agarose gel electrophoresis and visualized by exposure to ultraviolet light (302 nm).

SEQUENCES OF ISOLATES

As part of the experiment, the 18s rRNA gene from two

isolates of *Leishmania tropica* was sequenced. Using Blast and Bio edit, sequence alignment results were compared with data gathered from the gene bank, which is accessible online at NCBI.

CULTURE MEDIA

The parasites could be effectively separated from the dry, closed sores. After being extracted, the liquid and blood droplets were cultured in a semi-solid gelatinous culture medium that was packaged in a 25 ml bottle. After 24 hours of isolation, the sample was checked to make sure it was free of contamination after 4–7 days in a laboratory incubator set at 26°C.

After the cultures were inspected and the promastigotes was observed, 5.0 of the positive case bottles were transferred from the biphasic medium (NNN) into secondary culture media in order to sustain them. The medium (Roswell Park Memorial Institutes; RPMI1640) was utilized to multiply the promastigote and was incubated at a temperature of 26°C. Details specific to each animal (Meredith *et al.*, 1995) was applied to each vial, on the sixth day the promastigote parasites were extracted. Using a hemocytometer, the parasite count was adjusted to $1 \times 10^7/0.1$ ml for injection.

PLANT EXTRACTION PROCEDURE

PREPARATION OF AQUEOUS EXTRACT OF *CITRULLUS COLOCYNTHIS*

The seeds of *Citrullus colocynthis* were washed and bought from the nearby market. The University of Baghdad's Department of Biology conducted the identification of the seeds.

The colocynth plant was extracted using both alcoholic, hot and cold aqueous methods, using a Soxhlet extraction device and 400 ml of ethyl alcohol at a concentration of 95% for 24 hours, the materials were extracted from 20 grams of the dry material powder to create the alcoholic extract of the colocynth plant sample, following that the extract is dried at 40°C in an electric oven. following that the (Stock Solution prepared by adding 100 ml of distilled water to a solution made from 2 grams of the dry extract dissolved in a small amount of ethyl alcohol. As a result, the Stock Solution of 20 mg/ml was converted into concentrations of 5, 10, 15, and 20 mg/kg.

Citrullus colocynthis seed aqueous and alcoholic extracts were made in accordance with WHO (1998) and Harborne (1984). which involved taking 10 grams of the colocynth plant's dry matter powder and mixing it with 200 milliliters of cold distilled water. Using a magnetic mixer, it was combined with the solution for 15 minutes, and the mixture was then allowed to sit at room temperature for 24 hours. 0.22 Millipore filter paper was used to filter the

mixture. The suspended plant parts were then precipitated, and a clear solution was obtained by centrifuging the filtrate for 10 minutes at a speed of 300 times per minute. At 45°C, the clear solution was separated and dried in an electric oven. After that, the extract was weighed out of the dried powder and stored in the refrigerator until needed.

The method for preparing a boiled water extract was the same as before, with the exception that boiling water was utilized instead of distilled water to gauge the potency of the cocoa plant's boiled water extract. The following mg/kg concentrations (0.5, 1, 1.5, and 2%) were made: Additionally, a stock solution was made by combining two grams of the dry extract with a small amount of ethyl alcohol and adding 100 milliliters of distilled water to dilute it. This resulted in an original solution with a concentration of 20 mg/ml, from which concentrations of (5, 10, 15, and 20) mg/kg were obtained.

PREPARATION OF (AgNO₃) SOLUTION

After thorough washing, 400 milliliters of boiling distilled water were used to soak four grams of dried citrullus seeds overnight. When the extract was utilized to make the nanoparticles, it was centrifuged for seven minutes at 8000 rpm at room temperature and then filtered once more. Afterward 0.017 g of (AgNO₃) was dissolved in 100 ml of deionized distilled water to create 1 mM of AgNO₃ solution.

GREEN SILVER NANOPARTICLE SYNTHESIS

Synthesis of green silver nanoparticles: A colorless solution was obtained by vigorously swirling 0.001M silver nitrate into 50 ml of distilled water. The solution became dark when 6 milliliters of *Citrullus colocynthis* extract was added, and the mixture was rapidly swirled for 20 minutes at 75 degrees Celsius. This indicates that Ag ions had been reduced and green silver nanoparticles had been created by (Meena and Chouhan, 2015).

GREEN SILVER NANOPARTICLES: CHARACTERIZATION

UV-VISIBLE ABSORPTION AgNP SPECTROPHOTOMETER

UV-VIS double beam spectrophotometers from SHIMADZU were used to measure the absorbance spectra (SPE spectra) of the AgNPs solution. Every spectrum was recorded in a quartz cell with a 1 cm optical path at room temperature. As a blank, deionized distilled water was utilized. The range of absorption measured was 200–800 nm. According to Luis *et al.* (2014), certain samples were diluted 1:10 in deionized water since they had become excessively concentrated.

ATOMIC FORCE MICROSCOPE (AFM) ANALYSIS (NISHIDA *ET AL.*, 2008)

Under typical air circumstances, the surface morphology

of the nanoparticles was seen using an atomic force microscope in contact mode. AFM analysis was performed with an AA3000. After sonicating a sample of Ag NP solution with distilled water, a little drop of the sample was put on a glass slide measuring 2 by 1 cm and left to dry. Following that, the slide was put on the AFM sample stage, and the analysis was done in accordance with protocol.

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) ANALYSIS (AWWAD ET AL., 2012)

FTIR analysis (Shimadzu) was used to study the characterization of functional groups on the surface of AgNPs by plant extracts. The spectra were scanned between 4000 and 400 cm^{-1} at a resolution of 4 cm^{-1} . To construct the samples, the AgNPs were uniformly placed over a dry matrix of potassium bromide and compressed to form an almost transparent disc. The samples were analyzed using potassium bromide as the standard. During the analysis, every sample utilized in this study was in its original liquid state.

ANALYSIS USING SCANNING ELECTRON MICROSCOPY (SEM)

scanning electron microscope (SEM) was used to look at the formed nanoparticles. The morphological evaluation of the samples was carried out by the Ministry of Science and Technology using the Mira3 Tescan for SEM analysis. The samples were laid out on a slide and then coated with platinum using an auto fine coater. A review of the content was then carried out (Prasad et al., 2011).

EXPERIMENTS CONDUCTED IN VITRO

SKIN LEISHMANIASIS VITALITY TEST

The parasites vitality was evaluated using 0.4% Erythrocyhn-B dye diluted with phosphate buffer saline (PBS). After that, the mixture was cooled to 4°C and allowed to sit in ice for five minutes. Then, using a hemocytometer to determine the percentage of cell vitality, a drop of the mixture was inspected and at least 100 cells were counted. The computation made and five replicates were found, where the pigmented parasite indicates dead cells and the non-pigmented parasite indicates living cells.

Treatments applied to the hot and cold alcoholic and aqueous extracts of the colocynth plant in the parasite suspension cultured outside of the living body in the two-phase culture medium:

Using a counting slide, counting and conditioning treatments were applied to the parasites once they had matured and multiplied. There were approximately 400 promastigote per milliliter in the afore mentioned culture media. After determining the vital percentage at the start and finish of the experiment, roughly 0.5 mL

was transferred. Suspended parasites cultured in Roswell Park Memorial Institutes' (RPMII640) prepared culture medium (NNN)

In order to perform the experiment, various concentrations of the extract (0.5, 1, 1.5, 2%)/ml were applied to glass bottles that held the culture medium and the parasite suspension. For every concentration, there were five duplicates, with five bottles serving as the control group that were not treated.

At the start of the experiment, the arithmetic rate of vitality was determined using a dye guide (0.4% Erythrocyhn-B).

Each concentration was placed separately and then vigorously shaken to ensure the distribution and spread of the extract within the medium containing the parasites with the concentrations shown above in the extract to the incubator for preserving the parasites at 26 degrees Celsius. For ten days, the necessary counting operations were performed on it to determine the impact of the concentrations made from the extract from the exchange.

STATISTICAL ANALYSIS

The SAS (2018) software was utilized to ascertain the impact of variance variables on the study parameters. In this investigation, the least significant difference (LSD) was employed to compare means significantly (ANOVA/one way).

RESULTS AND DISCUSSION

Clinical assessment, ten of the fifty pets that had clinical examinations had clinical suspicions. Skin lesions (alopecia, dermatitis), weight loss, emaciation, and long, brittle nails were observed as clinical signs. The others appear to be in good health. Just two dogs tested positive for *Leishmania* spp., whereas a cat tested negative for *Leishmania* spp.

MOLECULAR STUDY

Out of all the specimens, two positive samples were identified as *L. tropica* based on PCR results, the 2 suspected isolates of *Leishmania* spp. were confirmed by PCR technique, amplification of genomic DNA was conducted by using two primers under ideal circumstances, *L. tropica* was successfully amplified for the anticipated 500 bp segment of the 18srRNA gene (Figure 1).

The sequencing analysis results were immediately uploaded to GenBank under the accession numbers MZ430513.1 and MZ430514.1. A collection of *Leishmania* kDNA sequences, including two sequences of *Leishmania* records, were obtained from GenBank for phylogenetic studies. The positive sample in this study was found to be closely

linked to the Iranian *L. tropica* kDNA with accession number HM004586.1, and it matched with *L. tropica* from Belgium (HG512919.1), Portugal (LC459349.1), and China (KU194929.1). This was determined by phylogenetic analysis (Figure 2).

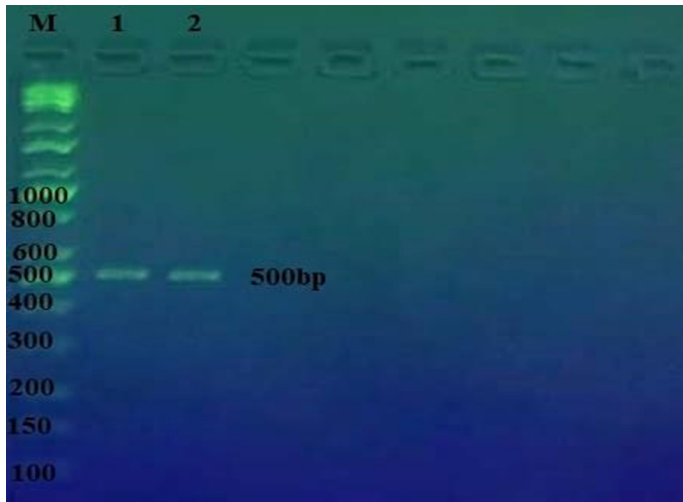


Figure 1: PCR product the band size 500 18SrRNA. The product was electrophoresed on 1.5% agarose at 70 volt/cm². 1x TBE buffer for 1:30 hours. N: DNA ladder (100), lane (1-2) represent (*Leishmania tropica*). PCR product of band size 500bp, visualized under U.V light .

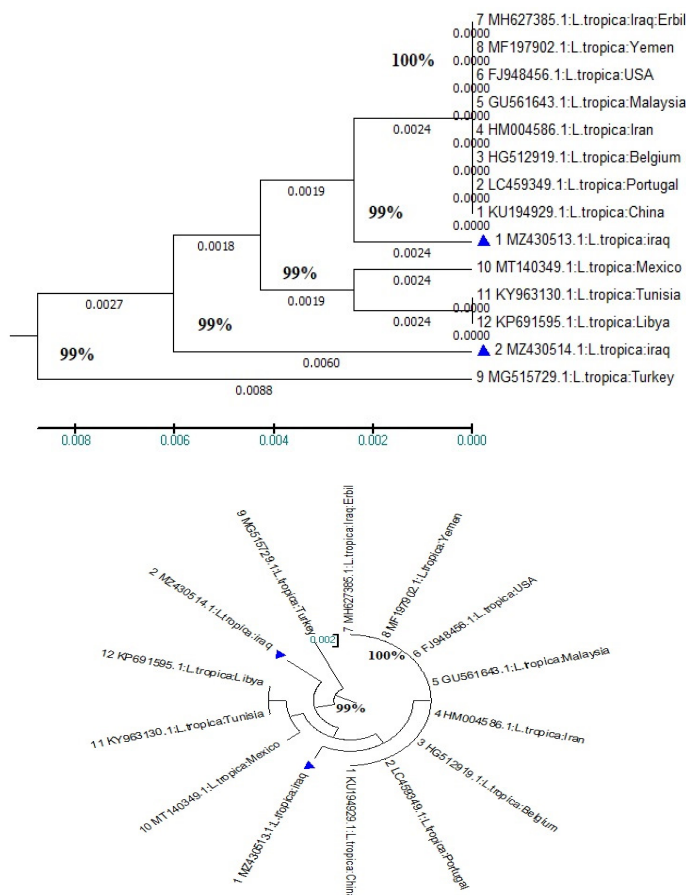


Figure 2: Phylogenetic analysis indicating genetic diversity between isolates based on kDNA sequence analysis.

SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES (AgNPs)

The preparation method of silver nanoparticles has been mentioned in our previous work in detail (Zein *et al.*, 2020).

The green approach was used to generate the silver nanoparticles, and UV-VIS spectroscopy, an atomic force microscope (AFM), Fourier transform infrared spectroscopy (FTIR), and a scanning electron microscope (SEM) were used to analyze the particles (Figure 3) illustrates how the aqueous seed extract of the colocynth plant functions as a reducing agent, converting metallic silver to nanosilver and producing the desired color shift. It is commonly known that silver nanoparticles in aqueous solution have a light brown color. This phenomenon may be related to the stimulation of the particles' surface plasmon vibrations. It is discovered that Ag²⁺ ions of silver nitrate are converted to Ago atoms (Thombre *et al.*, 2013; Krithiga *et al.*, 2015).

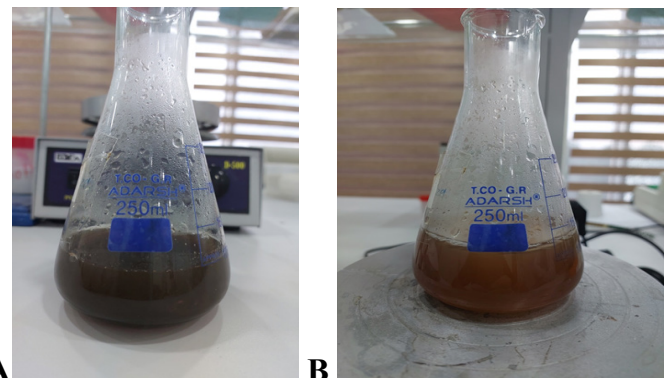


Figure 3: (A) *Citrullus colocynthis* Aqueous seed extract (B) green synthesis of AgNPs with *Citrullus colocynthis* aqueous seed extract after stirring 20min at 75 °C.

Determine the effect of different concentrations of alcoholic extract on the rate of number and viability of the promastigote.

As indicated in Table 1, the results demonstrated that there are significant differences between the treatment's effects at the (P<0.01)% probability level. When compared to the control group of 100, the concentration of 2 grams had the greatest significant effect on the eighth day, causing killed 100% of the live parasites. This was significantly different from the third day, when the promastigote vitality percentage was reduced to 55%. While the remaining concentrations (1, 1.5, and 0.5) demonstrated that the exposure times had a significant effect on the parasite's vitality after eight days, concentration 1.5 had the greatest effect, reducing the parasite's living percentage to 11%, concentration 1 to 10%, and concentration 0.5 to 7%. However, on the ninth day of treatment, all concentrations were able to completely kill the parasite.

Table 1: The effect of different concentrations of the alcoholic extract of the colocynth plant on the vitality rate of the promastigote stages during different periods of growth.

Conc. ()	Date										Mean $\pm$ SD
	1	2	3	4	5	6	7	8	9	10	
Control	100	100	100	100	100	100	100	100	100	100	100 $\pm$ 0.00 a
0.5	98	88	70	60	57	49	22	7	0	0	45.1 $\pm$ 37.52 b
1	96	75	65	45	33	29	12	10	0	0	36.5 $\pm$ 34.03 b
1.5	92	70	60	50	44	30	15	11	0	0	37.2 $\pm$ 31.42 b
2	85	65	55	40	30	25	4	0	0	0	30.4 $\pm$ 30.27 b
L.S.D. (P-value)											18.371 (0.0001)

Means having with the different letters in same column differed significantly. ** ($P \leq 0.01$).

Table 2: The effect of different concentrations of the aqueous hot extract of the colocynth plant on the vitality rate of the promastigote stages during different periods of growth.

Conc. ()	Day										Mean $\pm$ SD
	1	2	3	4	5	6	7	8	9	10	
Control	100	100	100	100	100	100	100	100	100	100	100 $\pm$ 0.00 a
0.5	95	95	95	95	95	95	95	95	95	90	94.5 $\pm$ 3.02 ab
1	96	96	96	96	96	96	96	96	96	94	95.8 $\pm$ 1.35 ab
1.5	90	90	90	90	90	90	90	90	90	88	89.8 $\pm$ 3.13 ab
2	85	85	85	85	85	85	85	85	85	80	84.5 $\pm$ 2.96 b
L.S.D. (P-value)											12.78 ** (0.0001)

Means having with the different letters in same column differed significantly. ** ($P \leq 0.01$).

Table 3: The effect of different concentrations of the aqueous cold extract of the colocynth plant on the vitality rate of the promastigote stages during different periods of growth.

Conc. ()	Day										Mean $\pm$ SD
	1	2	3	4	5	6	7	8	9	10	
Control	100	100	100	100	100	100	100	100	100	100	100 $\pm$ 0.00 a
0.5	95	95	95	95	95	95	95	95	95	93	94.8 $\pm$ 2.70 ab
1	96	96	96	96	96	96	96	96	96	95	95.9 $\pm$ 0.74 ab
1.5	90	90	90	90	90	90	90	90	90	90	90.0 $\pm$ 3.03 ab
2	85	85	85	85	85	85	85	85	85	83	84.8 $\pm$ 2.70 b
L.S.D. (P-value)											11.38 ** (0.0001)

Means having with the different letters in same column differed significantly. ** ($P \leq 0.01$).

EXTRACT OF AQUEOUS HOT AND COLD WATER COLOCYNTH PLANT

Understanding the beneficial effects of varying amounts of the colocynth plant's hot and cold water extract on the quantity and vitality of promastigote. The findings indicated that, for the same previous formulations, there were no significant differences under the ($P \leq 0.01$). probability level at concentration of (0.5, 1, and 1.5) , as shown in [Tables 2](#) and [3](#), where we did not observe any effect of the aqueous extract of the plant itself or any significant effect of the hot and cold aquatic colocynth extract on the vitality of promastigote. Only on the tenth day of treatment and the concentration of 2 grams had a slight significant decrease in the vitality of the promastigote.

Determine the effect of different concentration of green synthesis of silver nanoparticles of *Citrullus colocynthis* extract on the rate number and viability of promastigote:

As shown in [Table 4](#) there was significant decrease ($P \leq 0.01$) in number level rate and viability of ptomastgoid that treated with green AgNPs of *Citrullus colocynthis* comparing with control. It also was caused killing of the promastigote on third day after treatment in all concentration of green nanoparticles of plant (0.5, 1, 1.5 and 2) grams and shown best results in lowering viability in two days after treatment that was differ significantly from other alcoholic and aqueous cold and hot extract of plant.

Table 4: The effect of different concentrations of green synthesis of silver nanoparticles of *Citrullus colocynthis* extract on the rate number and viability of promastigote.

Conc. ()	Day										Mean ±SD
	1	2	3	4	5	6	7	8	9	10	
Control	100	100	100	100	100	100	100	100	100	100	100 ±0.00 a
0.5	95	95	0	0	0	0	0	0	0	0	19.0 ±49.86 b
1	96	96	0	0	0	0	0	0	0	0	19.2 ±39.19 b
1.5	90	90	0	0	0	0	0	0	0	0	18.0 ±38.19 b
2	85	85	0	0	0	0	0	0	0	0	17.0 ±35.92 b
L.S.D. (P-value)											27.67 ** (0.0001)

Means having with the different letters in same column differed significantly. ** (P≤0.01).

Since there are uncommon causes of cutaneous leishmaniosis in the old world, the isolation of *L. tropica* from dog skin lesions in a small number (2%), from the Baghdad governorate, is predicted, As reported by (Baneth *et al.*, 2017), *L. tropica* has been identified as the primary agent of cutaneous leishmaniosis in humans in the Middle East (Baneth *et al.*, 2016).

The effect of the of alcoholic extract of *Citrullus colocynthis* extract on the vitality and numbers of the Leishmania parasite *in vitro*.

It is clear from this study that the alcoholic extract of the colocynth plant has shown good effectiveness in influencing the vitality and growth of the promastigote stage of the *Leishmania parasite*. Where treatment of leishmaniasis with alcoholic extract of the colocynth plant at concentrations (0.5, 1.5, 2) grams per ml .There was a gradual and sharp decline in the vitality of the Leishmania parasite during the duration of the experiment. Where the concentration of 2 grams had the greatest significant effect on the eighth day, causing killed 100% of the live parasites. However, on the ninth day of treatment, all concentrations were able to completely kill the parasite. The reason for this may be attributed to the lethal effect of the alcoholic extract as a result of the release of some active substances from the bitter gourd plant that are included in its chemical composition as mount of phenolic acids (m-coumaric acid, gallic acid, vanillic acid) and the amount of flavonoids (quercetin) After conducting phytochemical screening on this plant, Uma and Sekar (2014) have identified alkaloids and saponins as the active components that may be responsible for its anthelmintic action; these compounds remained within the aqueous extract., which led to an effective effect on the vitality of the promastigoid of the leishmania parasite *in vitro* compared to the aqueous extract. Only on the tenth day of treatment and the concentration of 2 grams had a slight significant decrease in the vitality of the promastigote that did not contrasted to the study by *Citrullus columbthis* aqueous extract has been shown to have an inhibitory effect on the growth and

amount of protein and nucleic acids in the promastigote of tropical and visceral leishmaniasis, according to Jalal and Hassan's (2022) report on the possibility of using this extract as an alternative treatment for antileishmaniasis. The front phase was killed throughout the experiment (Tables 2 and 3). Sultan *et al.* (2010) and Khattari *et al.* (2020) have demonstrated that the plant contain in its composition substance called Colocynthitin which are a mixture of glycosides, alkaloids and an alcoholic substance called citrullol that make the plant toxic compounds against to many parasite (Sohani, 2021).

The effect of the green synthesis of silver nanoparticles of the colocynth plant on the vitality and numbers of the Leishmania parasite *in vitro*. At several doses (0.5, 1, 1.5, 2), the antileishmanial activity of silver nanoparticles against Leishmania tropica promastigotes was investigated (Table 4).

All above mentioned concentration were effective, strongly inhibiting the proliferation of, *L. tropica* promastigotes, and a shrinkage round shape appeared. It was cause killing the promastigotes on third day after treatment after 24 and 48 h of incubation comparing from other alcoholic and aqueous cold and hot extract of plant. A gradual decrease in the number of parasites appeared with increasing concentrations of plant each concentration of silver nanoparticles negatively affected the viability of *L. tropica* promastigotes. According to the majority of studies, the delayed release of silver ions from the nanoparticles' surface, which first degrade the cell's surface before penetrating the cytoplasm and binding with the target sites, may be the cause of the antileishmanial effects of the particles. Moreover, reactive oxygen species can be produced by silver nanoparticles. Since leishmania is well known to be extremely susceptible to these oxygen species, the medication that may produce ROS will be a highly effective antileishmanial (Zein *et al.*, 2022; Azimijou *et al.*, 2020; Awad *et al.*, 2020).

El-Khadragy *et al.* (2018) and Mohammed *et al.* (2019) have

shown the significant application of green manufactured AgNPs in their successful fight against *Leishmania parasites*. Green manufactured Ag nanoparticles have been shown by Hashemi *et al.* (2021) to be more efficient than glucantime at inhibiting the proliferation of parasite cell lines.

CONCLUSIONS AND RECOMMENDATIONS

Based on these finding , it is plausible to conclude that after two days of incubation, silver nanoparticles shown strong antileishmanial activity against *Leishmania tropica* promastigotes, even at low concentrations (0.5 mg). These findings may point to a different medication regimen in the future or suggest a complementary approach to treating leishmaniasis.

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NOVELTY STATEMENT

Using nanotechnology to treat afflicted animals with a novel leishmania parasite therapy offers up new therapeutic options for people.

AUTHOR CONTRIBUTION

AJZ handled parasitic isolation, identification, cultivation, and preservation and conducted the planning of the research performed PCR amplification, sequencing, data analysis, correspondence, and writing the paper, data curation, data recording, and. preservation of *Leishminal* isolates.

FUNDING

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ETHICS APPROVAL

This research project was approved by the Ethic Committee at Biotechnology Research Center, Al-Nahrain University.

CONSENT FOR PUBLICATION

The results of this research were approved author and agreed for publication.

AVAILABILITY OF DATA AND MATERIAL

Data, samples, and genomic materials used during this

study are available and stored at the Biotechnology Research Center, Al-Nahrain University.

CONFLICT OF INTEREST

This research was conducted without conflict of interest among authors, funding agencies, or with any other research group in other institutes.

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